植物研究雑誌
J. Jpn. Bot.
74: 344–352 (1999)

An Ultrastructural Study of Microspore Wall Morphogenesis in *Selaginella tamariscina* (Beauv.) Spring (Selaginellaceae)

Koichi UEHARA

Laboratory of Plant Morphology, Faculty of Horticulture,
Chiba University, Matsudo, Chiba, 271-8510 JAPAN

(Received on May 17, 1999)

The morphogenesis of the microspore wall in *Selaginella tamariscina* was examined using transmission electron microscopy. The sporoderm of *S. tamariscina* consists of three layers: the endospore, exospore, and perispore. The endospore is the thick, innermost layer, which has a low electron density. The exospore consists of three distinct sub-layers. The innermost sub-layer consists of several sheets of tripartite lamellae (lamellar layer). It is located on the proximal face of the microspore. The middle sub-layer is a uniformly thick, homogeneous layer (inner homogeneous layer). The outermost sub-layer is even thicker, conspicuously sculptured, and also consists of homogeneous material (outer homogeneous layer). The layers of the exospore begin to form just after meiosis in that order. The endospore forms at the same time as the outer homogeneous layer. Finally, the perispore is deposited on the exospore. The perispore is an electron-opaque layer that is tightly attached to the surface of the exospore. The endospore and the exospore lamellar layer appear to be derived from the cytoplasm of the microspore, while the inner and outer homogeneous layers of the exospore and the perispore are derived from the tapetum.

Key words: *Selaginella tamariscina*, microspore, sporoderm, exospore, perispore

Introduction

Pettitt (1966) reported the first transmission electron microscopy description of the morphology of the microspore of *Selaginella*. He described a homogeneous or faintly fibrous exine, without a lamellar layer, in the acetolysed microspore wall of *S. pulcherrima*. Gullvåg (1971) observed the lamellate layer in the exine of *S. selaginoides*. He considered that the exine of this species was derived from tapetal cells. Robert (1971a, 1971b, 1971c) described the morphogenesis of microspore walls of *S. kraussiana* and *S. selaginoides*. He observed two distinct layers (feuillet externe and feuillet interne) in the microspore of *S. selaginoides* (Robert 1971b, 1971c). Lugardon (1972) reported the morphology of mature microspore walls of *S. denticulata* and *S. selaginoides*. The stratification of the microspore walls in these two species is different. The microspore wall of *S. denticulata* has a perispore, a homogeneous outer exospore, and a lamellated inner exospore, while that of *S. selaginoides* has a para-exospore and a two-layered exospore. The para-exospore is equivalent to the feuillet externe of Robert (1971b). Tryon and Lugardon (1978) reported the morphology of microspores and megaspores of *S. galeottii*

and *S. martensii*. Microspores of these species have a perispore, a two-layered exospore, and an endospore. Lugardon (1978) also described the characteristics of three species of *Selaginella* (*S. denticulata*, *S selaginoides*, and *S. kraussiana*). He reported that the exospore of *Selaginella* consists of two distinct sub-layers: an outer astructured layer and an inner lamellated layer.

The composition of the microspore wall and its structure and stratification are different in each species of *Selaginella* that has been described. The purpose of this study was to examine the structure, development, and origin of the microspore wall of *S. tamariscina*, to compare these characters with those of other species of *Selaginella*, and to discuss the diversity of these characters.

Materials and Methods

Microsporangia of *Selaginella tamariscina* (Beauv.) Spring were collected at various developmental stages. They were fixed for 24 hr in 2 % paraformaldehyde – 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at 4°C (cf., Karnovsky 1965). After washing in the same buffer, they were postfixed in buffered 1 % osmium tetroxide for 2 hr. Subsequently, they were dehydrated through an ethanol series (30, 50, 70, 80, 90, 95, 100 %) and then in propylene oxide. Then, they were embedded in Spurr's epoxy resin (Spurr 1969). Sections were cut on a Reichert Ultracut-E ultramicrotome and stained with conventional uranyl acetate and lead citrate. Sections were examined under a JEOL JEM-100CX electron microscope at 80 kV.

Results

Tetrad stage

The first sporoderm formed is the exospore. It consists of three distinct sub-layers, the lamellar layer, and the inner and outer homogeneous layers.

After meiotic division, the spherical microspore mother cells divide to form the tetrahedral tetrad (Fig. 1). Each tetrad is enclosed by a low-electron-dense layer called the sporocyte coat (Pettitt and Jermy 1974). No sporoderm is yet formed on the microspore surface at this stage. A one-cell-thick secretory tapetum attaches to the inner surface of the sporangial wall cells (Fig. 2).

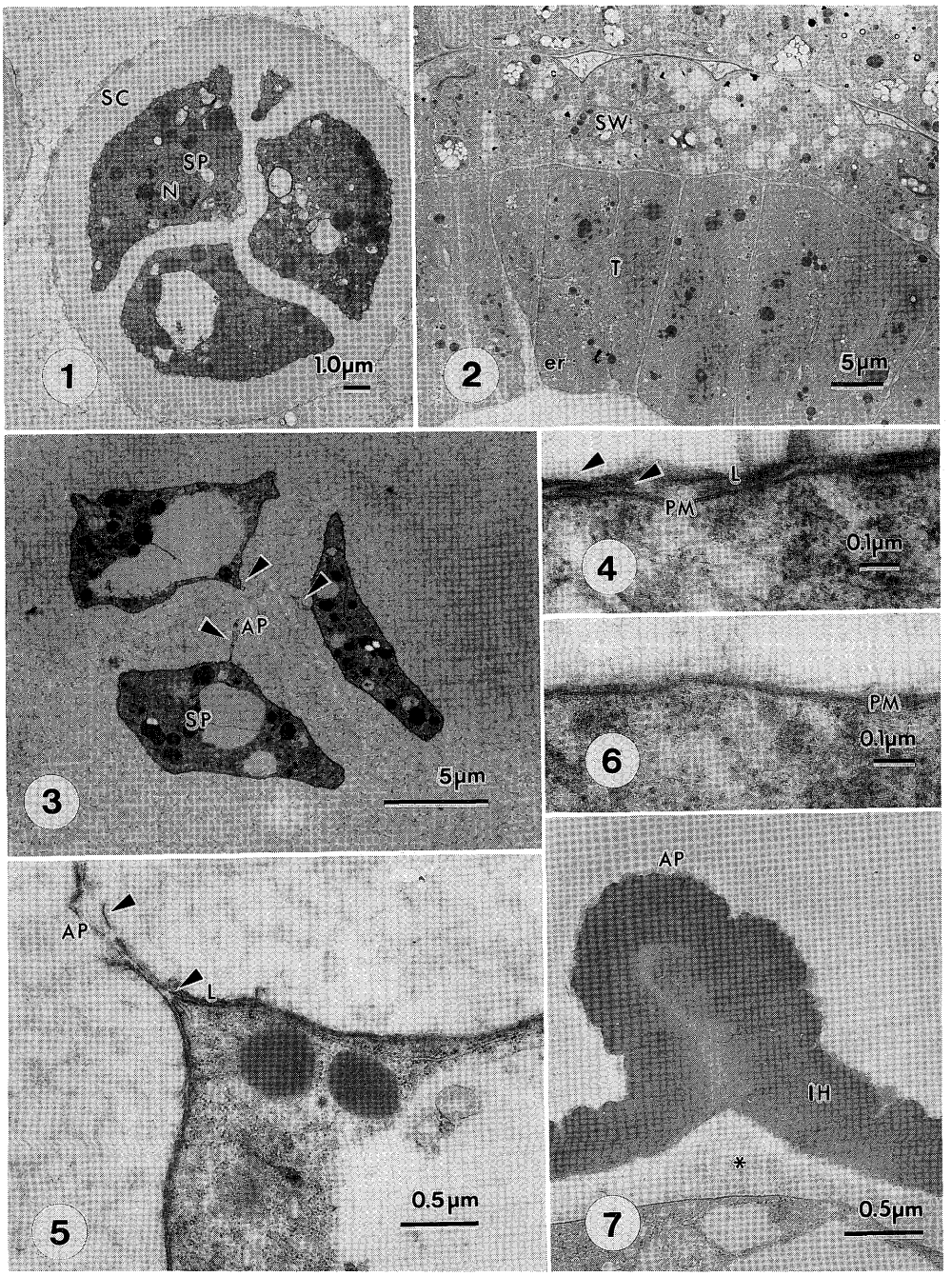
At the next stage, the projection of the initial aperture has appeared on the proximal face of each microspore (Fig. 3, arrowheads). The initial sporoderm begins to form on the surface of the microspore at this stage. Two to four sheets of tripartite lamellae are formed on the microspore plasma membrane on the proximal face (Fig. 4, arrowheads). This is the first stage of formation of the exospore lamellar layer. The initial aperture also consists of perpendicularly arranged tripartite lamellae (Fig. 5, arrowheads). These tripartite lamellae are located on the proximal, but not on the distal, surface of the microspore plasma membrane (Fig. 6).

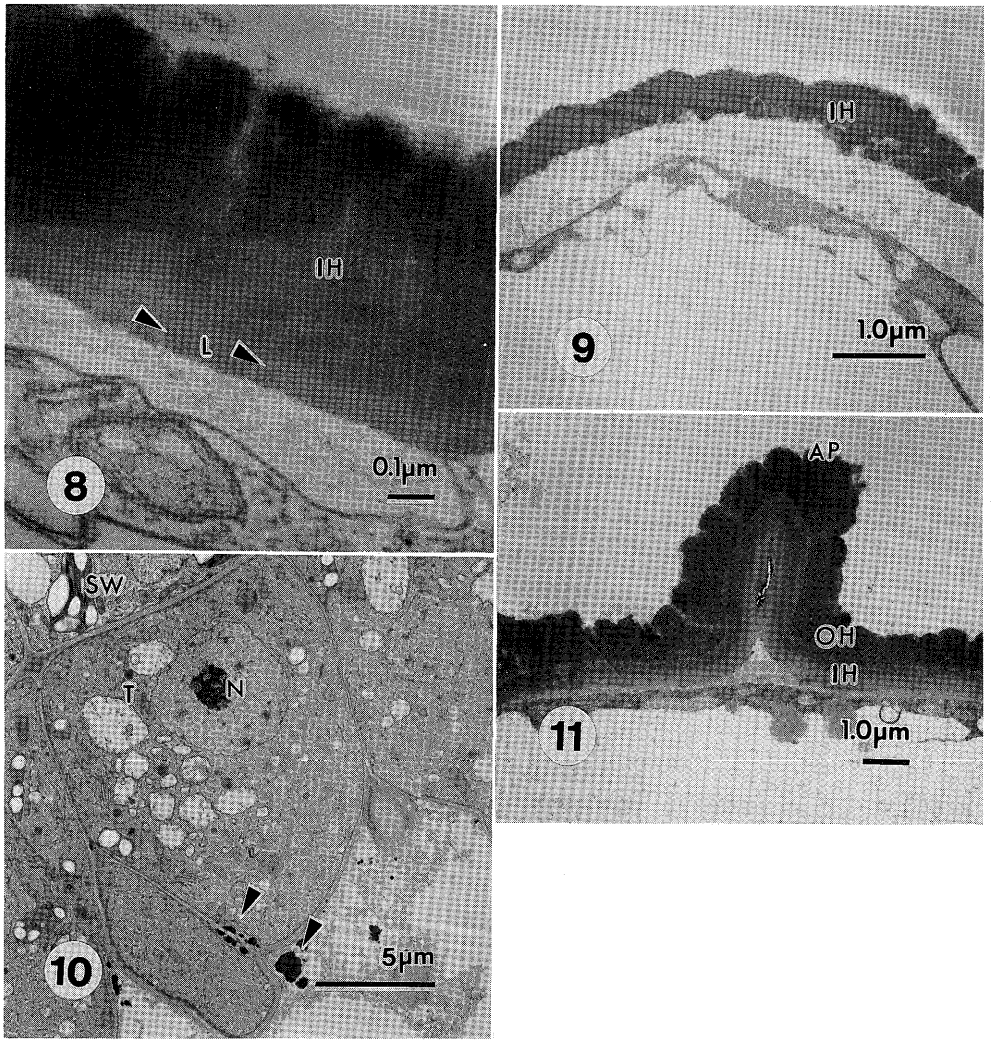
Next, a new sporoderm sub-layer is deposited on the microspore surface. This sub-layer is the inner homogeneous layer of the exospore (Fig. 7). It is 0.3–0.5 μm thick and consists of homogeneous material. On the proximal face, this layer is deposited on the lamellar layer (Fig. 8). Five to seven sheets of lamellae (exospore lamellar layer) can be observed under the inner homogeneous layer at this time (Fig. 8, arrowheads). The lamellar layer is observed on the proximal surface, but not on the distal face. Only the inner homogeneous layer is seen on the distal face at this stage (Fig. 9). Fibrillar material fills the space between the plasma membrane of the microspore and the developing exospore (Fig. 7, asterisk). The tapetal cytoplasm is active, and endoplasmic reticulum and dictyosomes are conspicuously developed. Electron-dense materials are ob-

served on the surface of the tapetum (Fig. 10, arrowheads).

Free-spore stage

An electron-dense outer homogeneous layer begins to be deposited on the surface of the inner homogeneous layer (Figs. 11, 12). The outer homogeneous layer is more electron dense than the inner one (Figs. 11, 12). By now, the lamellar layer of the proxi-





Figs. 8–11. Formation of the inner and outer homogeneous layers of the exospore. Fig. 8. High-magnification micrograph of the proximal face at the same stage as in Fig. 7. Several sheets of lamellae are seen under the developing inner homogeneous layer (arrowheads). Fig. 9. The inner homogeneous layer of the exospore is also deposited on the distal face as a discrete mass. Fig. 10. The tapetum in the exospore formation stage. Electron opaque material is observed on the surface of the tapetal cells (arrowheads). Fig. 11. Early free-spore stage. The outer homogeneous layer of the exospore is deposited on the inner homogeneous layer.

Figs. 1–7. Formation of the lamellar and inner homogeneous layers of the exospore. Fig. 1. Tetrad just after meiosis. An electron-lucent sporocyte coat encloses the microspores of the tetrad. Fig. 2. A one-cell-thick secretory tapetum is attached to the sporangial wall cells. Fig. 3. Formation of the exospore lamellar layer. Projections of the initial aperture appear on the proximal face of the tetrad (arrowheads). Fig. 4. Some sheets of tripartite lamellae are deposited on the proximal face of the plasma membrane (arrowheads). Fig. 5. The initial aperture also consists of tripartite lamellae (arrowheads). Fig. 6. There are no tripartite lamellae on the plasma membrane of the distal face. Fig. 7. Formation of the exospore inner homogeneous layer. Homogeneous material of moderate electron density is deposited on the proximal surface. A space that contains loose fibrous material is seen between the microspore plasma membrane and the developing exospore (asterisk). Figure Abbreviations: AP, aperture; CW, cell wall; EN; endospore; IH, Inner homogeneous layer; L, Lamellar layer; N, nucleus; O, orbicule; OH, Outer homogeneous layer; PE, perispore; PM, plasma membrane; SC, sporocyte coat; SP, microspore; SW, sporangium wall cell; T, tapetum.

mal face is almost indistinct (Fig. 13). The outer homogeneous layer of the distal face is thicker than that of the proximal face (Fig. 12). The cytoplasm of the microspore is vacuolated and its volume has increased. Some radial canals appear in the exospore (Fig. 12, arrowheads), and some orbicules, in the microsporangium (Fig. 13, arrowheads). The electron density of the orbicules is similar to that of the exospores outer homogeneous layer. The endospore is now evident under the exospore (Fig. 13).

The radial canals become more conspicuous in the exospore (Fig. 14, arrowheads), and the initial perispore, fibrous in nature, appears on the sculptured exospore (Fig. 14). The endospore is thin and still underdeveloped on the distal face. The tapetum has completely degenerated, and only the tapetal cell walls remain (Fig. 15).

The electron density of the perispore increases at its completion. The endospore thickens and is then complete (Fig. 16). The electron density of the completed outer homogeneous layer decreases, but it remains higher than that of the inner homogeneous layer (Fig. 16). The electron density of the orbicules decreases, as in the exospore outer homogeneous layer (Fig. 17). A perispore-like structure is also deposited on the orbicules (Fig. 17, arrowheads). After completion of the exospore, the outer homogeneous layer becomes fully sculptured.

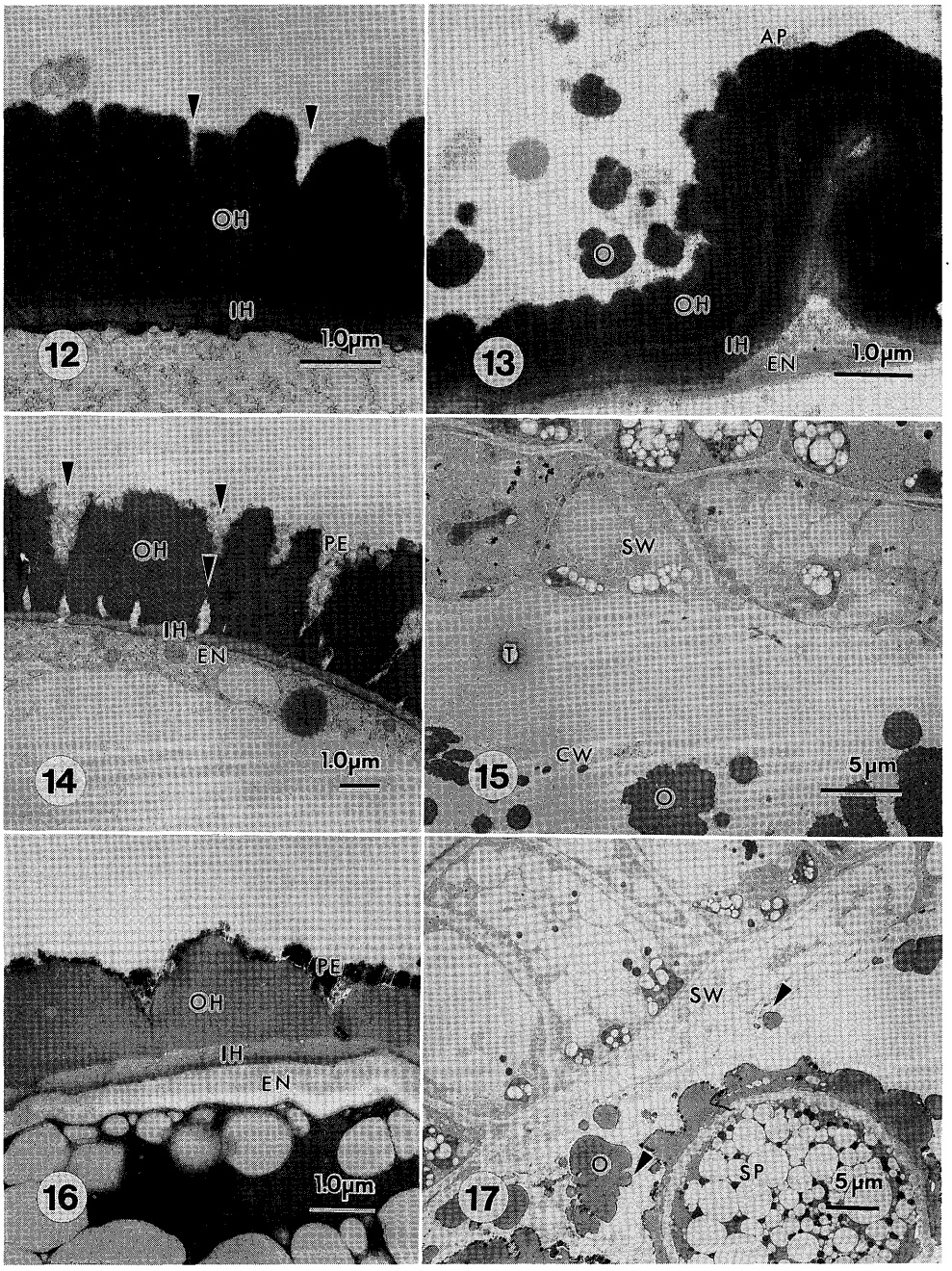
Discussion

The process of sporoderm formation in *S. tamariscina* is summarized in Fig. 18. In this study, the exospore of *S. tamariscina* was found to consist of 3 distinct sub-layers: the lamellar layer, and the inner and outer homogeneous layers. The lamellar layer consists of several sheets of tripartite lamellae. The lamellated sub-layer of exospore is widely observed in the exospores of pteridophytes (Uehara and Kurita 1989b, 1991, Uehara et al. 1991, Tryon and Lugardon 1991). It is similar to the endexine lamellae of Gymnosperm and Angiosperm pollen (Rowley 1995). These tripartite lamellae form centripetally and are considered to be of gametophytic spore origin (Uehara and Kurita 1986, 1989b, 1991; Uehara et al. 1991; Tryon and Lugardon 1991). Although the inner and outer homogeneous layers of the exospore and the perispore are deposited centrifugally, they are derived from the tapetum (Fig. 18). The electron-dense perispore of *S. tamariscina* is similar to the perispores generally observed in other groups of pteridophytes, such as in the Filiceae (Lugardon 1971, 1978) and *Isoetes* (Uehara et al 1991).

The exospores of *Selaginella* that have already been described are generally recognized as having distinct inner and outer sublayers. The inner layer consists of imbricate lamellae and is not very electron dense. It is tangentially divided into several elements

Figs. 12–17. Formation of the exospore outer homogeneous layer, endospore, and perispore. Fig. 12. The outer homogeneous layer of the exospore is also deposited on the distal face. The electron density of the outer homogeneous layer is higher than that of the inner one. Some radial canals are observed in the outer homogeneous layer (arrowheads). Fig. 13. Orbicules appear around the developing microspore, and the endospore appears under the exospore. Fig. 14. Formation of the perispore. The thick, sculptured, outer homogeneous layer has been completed. Conspicuous radial canals have appeared in the exospore (arrowheads). An initial fibrous perispore appears on the exospore. The endospore is still underdeveloped on the distal face. Fig. 15. The tapetum has degenerated and only tapetal cell walls remain. Fig. 16. Completed sporoderm. The electron density of the exospores outer homogeneous layer decreases. The endospore and electron-opaque perispore have thickened and are now complete. Fig. 17. Overview of the mature microsporangium. A perispore-like structure is deposited on the surface of the orbicules (arrowheads).

designated as the fine laminated zone (Tryon and Lugardon 1991) in *S. selaginoides*, *S. denticulata* and *S. martensii* (Lugardon 1978; Tryon and Lugardon 1978). This zone appears on both sides of the aperture. The outer layer consists of amorphous sporopollenin (Tryon and Lugardon 1991). The outer homogeneous layer of *S. tamariscina* is equivalent to the outer layer of other *Selaginella* exospores. The inner homogeneous and lamellar layers become indistinct during their development and appear to be a single



layer at maturity. The combined lamellar and inner homogeneous layers of *S. tamariscina* are equivalent to the inner layer of other *Selaginella* exospores. In the inner layer of other *Selaginella*, the lamellae may mingle with the homogeneous material. Tryon and Lugardon (1978) described how the inner layer appeared homogeneous with granules on its distal face and showed lamellae at the apertural ridge in *S. martensii*. In *S. tamariscina*, the lamellar and inner homogeneous layers apparently form independently. The exospore stratification of *S. tamariscina* is thus fundamentally equivalent to that of other species of *Selaginella*.

In other groups of pteridophytes, the exospore mainly consists of two sub-layers, an inner lamellated layer and an outer homogeneous layer (Lugardon 1971, 1976, 1979; Uehara and Kurita 1986, 1989b; Uehara et al. 1991). The spores of *Equisetum* (entirely homogeneous) and *Lycopodium* (entirely lamellar) are exceptions (Uehara and Kurita

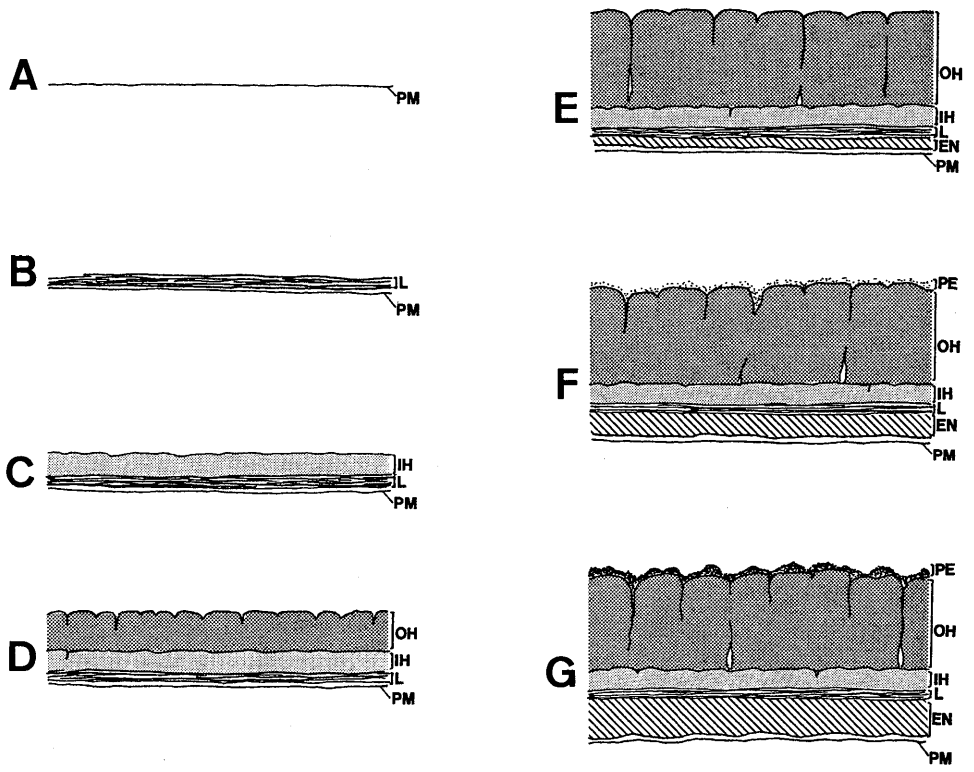


Fig. 18. Formation of the sporoderm in *Selaginella tamariscina*. A. Just after meiosis, no sporoderm has yet formed on the microspore plasma membrane. B. Formation of the exospore lamellar layer. Several sheets of tripartite lamellae appear on the proximal face of the microspore plasma membrane. C. Formation of the exospore inner homogeneous layer. Uniformly thick homogeneous material is deposited all over the microspore's surface. D. Formation of the exospore outer homogeneous layer. Electron-dense homogeneous material is deposited on the inner homogeneous layer. E. The outer homogeneous layer of the exospore has thickened and become sculptured. The initial endospore appears beneath the proximal face of the exospore. F. Formation of the perispore. The initial fibrous perispore has been deposited on the exospore. G. Completed sporoderm. The endospore has thickened. The perispore becomes a condensed, electron-opaque layer.

1989a, 1991). The author considers that the exospore of *Selaginella*, consisting of the inner lamellae and outer homogeneous material, is homologous to the exospore of the pteridophytes.

The exospore of other Lycopsida, *Lycopodium* and *Isoetes*, consists mainly of lamellae. Some *Selaginella* microspores (*S. selaginoides*, *S. denticulata* and *S. martensii*) also have a lamella-rich exospore. In particular, the microspore of *S. selaginoides* has a para-exospore, which consists of lamellar and homogeneous materials similar to the *Isoetes* microspore. On the other hand, the exospores of *S. kraussiana*, *S. galeottii* and *S. tamariscina* consist of mostly homogeneous material and have few lamellae. The diversity of the exospores of *Selaginella* species is based on the number of lamellae, and the presence or absence of the fine laminated zone and para-exospore. There is a complementary relationship between the number of lamellae and the amount of homogeneous material.

Variation in the number of lamellar layers occurs in the exospores of ferns. Primitive eusporangiate ferns have many lamellae; whereas advanced leptosporangiate ferns tend to have fewer (Lugardon 1971). The number of lamellae varies from 10 to 12 in less-derived genera such as *Ophioglossum*, to a single lamella in the spores of derived ferns like *Blechnum* (Tryon and Lugardon 1991). The author believes that the lamella-rich exospore of *Lycopodium*, *Isoetes*, and some of the *Selaginella* species is a primitive type, while an exospore with few lamellae, such as those of *S. tamariscina*, is a derived character. In other groups of pteridophytes, variation in exospore stratification occurs at the family or order level, and is stable at the genus or species level. However, the exospore stratification is diverse within the genus *Selaginella*.

References

Gullvåg B. M. 1971. The fine structure of some pteridophyte spores before and during germination. *In*: Erdtman G. and Sorsa P. (eds.), Pollen and Spore Morphology/Plant Taxonomy. Pteridophyta, pp. 252–295. Almqvist & Wiksell, Stockholm.

Karnovsky M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**: 137A–138A.

Lugardon B. 1971. Contribution à la connaissance de la morphogénèse et de la structure des parois sporales chez Filicinées isosporées. Thesis, University of P. Sabatier, Toulouse.

—— 1972. Sur la structure fine et la nomenclature des parois microsporales chez *Selaginella denticulata* (L.) Link et *S. selaginoides* (L.) Link. Compt. Rend. Hebd. Séances Acad. Sci. **274**: 1656–1659.

—— 1976. Sur la structure fine de l'exospore dans les divers groupes de Ptéridophytes actuelles (microspores et isospores). *In*: Ferguson I. K. and Muller J. (eds.), The Evolutionary Significance of the Exine, Linnean Society Symposium Series 1, pp. 231–250. Academic Press, London.

—— 1978. Isospore and microspore walls of living pteridophytes: identification possibilities with different observation instruments. *In*: Proc. of IV Int. Palynol. Conf., Lucknow (1976–1977), pp. 152–163.

—— 1979. Sur la formation du sporoderme chez *Psilotum triquetrum* Sw. (Psilotaceae). Grana **18**: 145–165.

Pettitt J. M. 1966. Exine structure in some fossil and recent spores and pollen as revealed by light and electron microscopy. Bull. Brit. Mus. Nat. Hist. Geol. **13**: 223–257.

—— and Jermy A. C. 1974. The surface coats on spores. Biol. J. Linn. Soc. **6**: 245–257.

Robert D. 1971a. Nouvelle contribution à l'étude de l'origine des parois microsporales chez le *Selaginella kraussiana* A. Br. Compt. Rend. Hebd. Séances Acad. Sci. **272**: 385–388.

—— 1971b. Etude, en microscopie électronique, des modalités d'édification des parois microsporales chez le *Selaginella selaginoides* (L.). Mise en place du feuillet externe. Compt. Rend. Hebd. Séances Acad. Sci. **273**: 332–335.

—— 1971c. Etude, en microscopie électronique, des modalités d'édification des parois microsporales chez le *Selaginella selaginoides* (L.). Mise en place du feuillet interne. Compt. Rend. Hebd. Séances Acad. Sci. **273**: 1933–1935.

Rowley J. R. 1995. Are the endexines of pteridophytes, gymnosperms and angiosperms structurally equivalent? Rev. Palaeobot. Palynol. **85**: 13–34.

Spurr A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. **26**: 31–43.

Tryon A. F. and Lugardon B. 1978. Wall structure and mineral content in *Selaginella* spores. Pollen Spores **20**: 315–340.

—— and —— 1991. Spores of the Pteridophyta. Springer-Verlag, New York.

Uehara K. and Kurita S. 1986. Ultrastructure of the sporoderm morphogenesis in *Psilotum nudum* (L.) Griseb. Res. Inst. Evolut. Biol. Sci. Rep. **3**: 171–181. In Japanese.

—— and —— 1989a. An ultrastructural study of spore wall morphogenesis in *Equisetum arvense*. Amer. J. Bot. **76**: 939–951.

—— and —— 1989b. Ultrastructural study of spore wall morphogenesis in *Ophioglossum thermale* Kom. var. *nipponicum* (Miyabe & Kudo) Nishida. Bot. Mag. Tokyo **102**: 413–427.

—— and —— 1991. Ultrastructural study on spore wall morphogenesis in *Lycopodium clavatum* L. (Lycopodiaceae). Amer. J. Bot. **78**: 24–36.

——, ——, Sahashi N. and Ohmoto T. 1991. Ultrastructural study on microspore wall morphogenesis in *Isoetes japonica* (Isoetaceae). Amer. J. Bot. **78**: 1182–1190.

上原浩一：イワヒバの小胞子壁形成過程の超微形態学的研究

イワヒバの小胞子壁の形態形成過程を透過型電子顕微鏡により観察した．小胞子壁は内側から内膜・外膜・周皮の3つの構造からなっていた．外膜には3つの層構造が観察され内側からラメラからなる外膜ラメラ層（向心極側のみに存在），均質なホモジニアス内層・ホモジニアス外層からなっていた．外膜のホモジニアス外層は厚く不均一で胞子の彫紋を形作っていた．小胞子壁は減数分裂の後，外膜の内側の層（ラメラ層）から形成されホモジニアス内層・外層の順で堆積した．内膜は外膜のホモジニアス外層と同時期に外膜の内側に堆積した．周皮は外膜形成後，さらに外側に堆積した．観察結果から内膜と外膜ラメラ層は小胞子細胞により作られるが，外膜ホモジニアス層（内層・外層）および周皮はタペータムに由来すると考えられた．

（千葉大学園芸学部）